Report for 2001AZ901B: Measurement of Hormonal Activity and Volume Contribution of Treated Wastewater in Water from Wells Along the Santa Cruz

There are no reported publications resulting from this project.

Report Follows:

A. Problem and Research Objectives

Even full utilization of the Tucson regional allotment of Central Arizona Project water (approximately 140,000 acre-feet per year for the City of Tucson) will not indefinitely satisfy area water requirements, without resorting to groundwater mining. Consequently, water reuse will become an increasingly important part of regional water supply planning in decades to come. Only the designation of permissible uses for reclaimed water and regulatory issues remain in doubt. Currently, about 10,000 acre-feet (AF) of reclaimed water is infiltrated and recovered for reuse each year at the Sweetwater Recharge Facilities (SRF) in west Tucson. The facility is operated by the City of Tucson, but receives chlorinated secondary effluent from Pima County's Roger Road Wastewater Treatment Plant (RRWTP). Recovered water is used for landscape irrigation. RRWTP effluent above the 10,000 AFY that is infiltrated and recovered is discharged to the Santa Cruz River (Figure 1) where, during perhaps 11 months of the year, it percolates into the (otherwise) dry streambed or is lost due to evapotranspiration. Effluent from Pima County's Ina Road Wastewater Treatment Plant is also discharged into the Santa Cruz, about 5 miles downstream from the RRWTP outfall. Together, the plants provide an average of 50,000 AFY to the river, much of which recharges the Tucson aquifer. Gaylean (1996) estimated that about 90% of the effluent discharged to the Santa Cruz infiltrates in the 23-mi reach between the Roger Road treatment facility and Trico Road. Estimates of infiltration values for effluent releases on the Santa Cruz River (compiled in Lacher, 1996) vary from 5.0 to 8.7 ac-ft/mi/day. The degree to which reclaimed waters contribute to local well waters and affect quality characteristics in potable wells along the Santa Cruz River is not known.

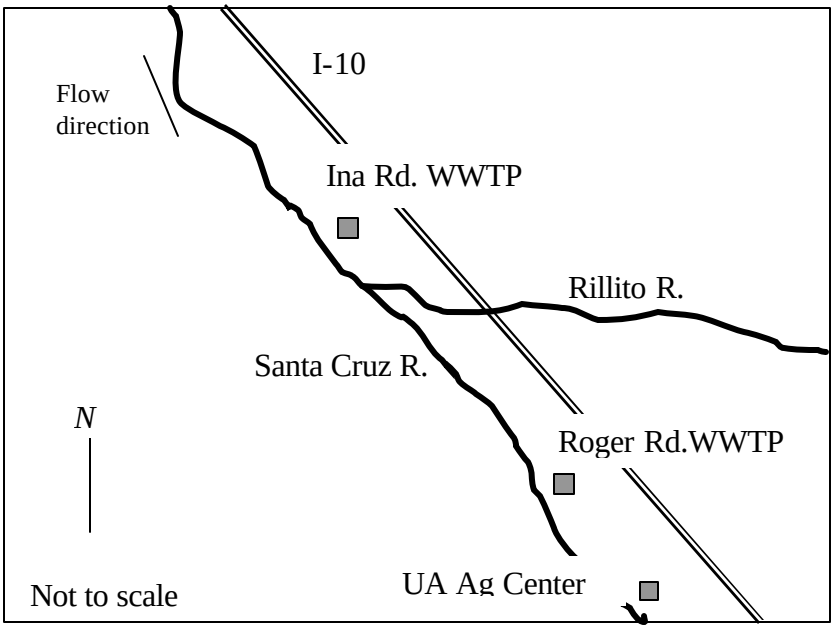

Figure 1. Schematic of Santa Cruz River study area and landmark locations.

The presence of wastewater effluent among waters withdrawn from wells along the Santa Cruz downstream from the Roger Road and Ina Road Wastewater Treatment Plants has never before been comprehensively examined. A few previous studies documented the presence of wastewater effluent in ground waters extracted from isolated wells along the Santa Cruz. Using stable oxygen isotopes ($^{18}O/^{16}O$), Bostick (1978) estimated that water obtained from a City of Tucson well (Z-1) located ½ mile from the RRTP contained 30% native ground water and 70% recharged wastewater. Similarly, Leenhouts (1998), using boron isotopes ($^{10}B/^{11}B$), calculated that an irrigation well in the Cortaro-Marana Irrigation District contained 75% native ground water and 25% municipal wastewater at the beginning of an irrigation season. The percentage of irrigation return flow in well water increased during the irrigation season.

Wastewater effluent contains traces of many compounds that are added during water use. A number of pharmaceutically-active compounds (PhACs) are known to survive secondary treatment and to persist in surface waters influenced by effluent disposal (Kolpin *et al*., 2002). Although previous studies indicated that bulk organics are efficiently removed during percolation through surface soils and sediments (Wilson *et al*., 1995; Quanrud *et al*., submitted), the fate of PhACs during the infiltration of effluent is poorly understood.

A variety of known estrogenic compounds elicit measurable ecological changes at or below nanomolar concentrations in water. These compounds afford analytical challenges when presented in a complex aqueous matrix at those levels. Furthermore, it is not possible to anticipate estrogenic effects due to the many, largely uncharacterized, trace organic residuals in treated wastewater. Consequently, *in vitro* tests were used to measure estrogenic activity in whole-water samples and organic concentrates derived from those water samples.

In this project, we established the volume contribution of reclaimed water in wells proximate to the Santa Cruz and explored the relationships between fractional effluent content, dissolved organic carbon (DOC) concentration and the results of *in vitro* measurements of estrogenic hormonal activity in a contemporary set of water samples. Boron and boron isotope measurements were used to establish the volume contribution of wastewater effluent in monitoring wells along the Santa Cruz River. Volume fraction of wastewater origin and estrogenic activity were correlated in the same set of samples. Results suggest that incidental recharge of effluent in the Santa Cruz River bed affects the quality of proximate ground waters.

Endocrine Disruptors: Literature Review

Endocrine disrupting compounds (EDCs) include a large number of natural and synthetic hormones, pharmaceuticals, pesticides, and industrial/household chemicals. EDCs have been detected in wastewater. Some resist biochemical degradation, persist in the environment, and bioaccumulate. EDCs in the environment can adversely affect wildlife and may be associated with human health effects.

A number of observable ecological effects arise from exposure to EDCs in aquatic environments. In the United Kingdom, for example, vitellogenin, an egg precursor protein, was detected in male trout and other fishes immediately downstream from the outfalls of wastewater treatment plants (Jobling *et al*., 1998). Wastewater-derived compounds that have been implicated as a source of such effects include human estrogen and its metabolites, pharmaceutical estrogens and alkylphenols from industrial cleaners (Desbrow *et al*., 1998; Snyder *et al*., 1999,2001).

Because chemicals interfere with endocrine system function in several ways, it is probable that no single assay can predict the nature and extent of hormone-disrupting activities by mixtures of compounds or wastes. *In vitro* assays for estrogenic activity that have received attention is this regard include receptor binding, reporter gene, and cell proliferation assays. However, test-dependent sensitivities to aqueous-phase 17β-estradiol (E2), the primary human estrogen, differ by orders of magnitude. Assays also require very different levels of effort and time. The receptor-binding (ligand displacement) test used here is particularly fast and requires no extraordinary skills to perform.

The receptor-binding assay is based on displacement of a fluorescent ligand from a human estrogen receptor (ER-β) as a measure of estrogenic activity in water samples or concentrates derived from water samples. The same test was used previously in our laboratory to show that estrogenic compounds surviving secondary treatment are substantially removed during the managed infiltration of domestic wastewater effluent for groundwater replenishment (Turney *et al*., 2002).

B. Methodology

Water samples were collected from shallow wells located along the Santa Cruz River starting at the University of Arizona's West Campus Agricultural Center just upstream (south) of the SRF and continuing downstream well beyond the outfall of the Ina Road Wastewater Treatment Plant (Figure 2). Sampling points provided waters with significantly different reclaimed water content, due primarily to screened interval (Figure 3) and distance from Roger Road and Ina Road Wastewater Treatment Plant outfalls. Surface water samples were collected from the Santa Cruz River at the points indicated in Figure 2. These were subjected to the same battery of analyses as the ground waters.

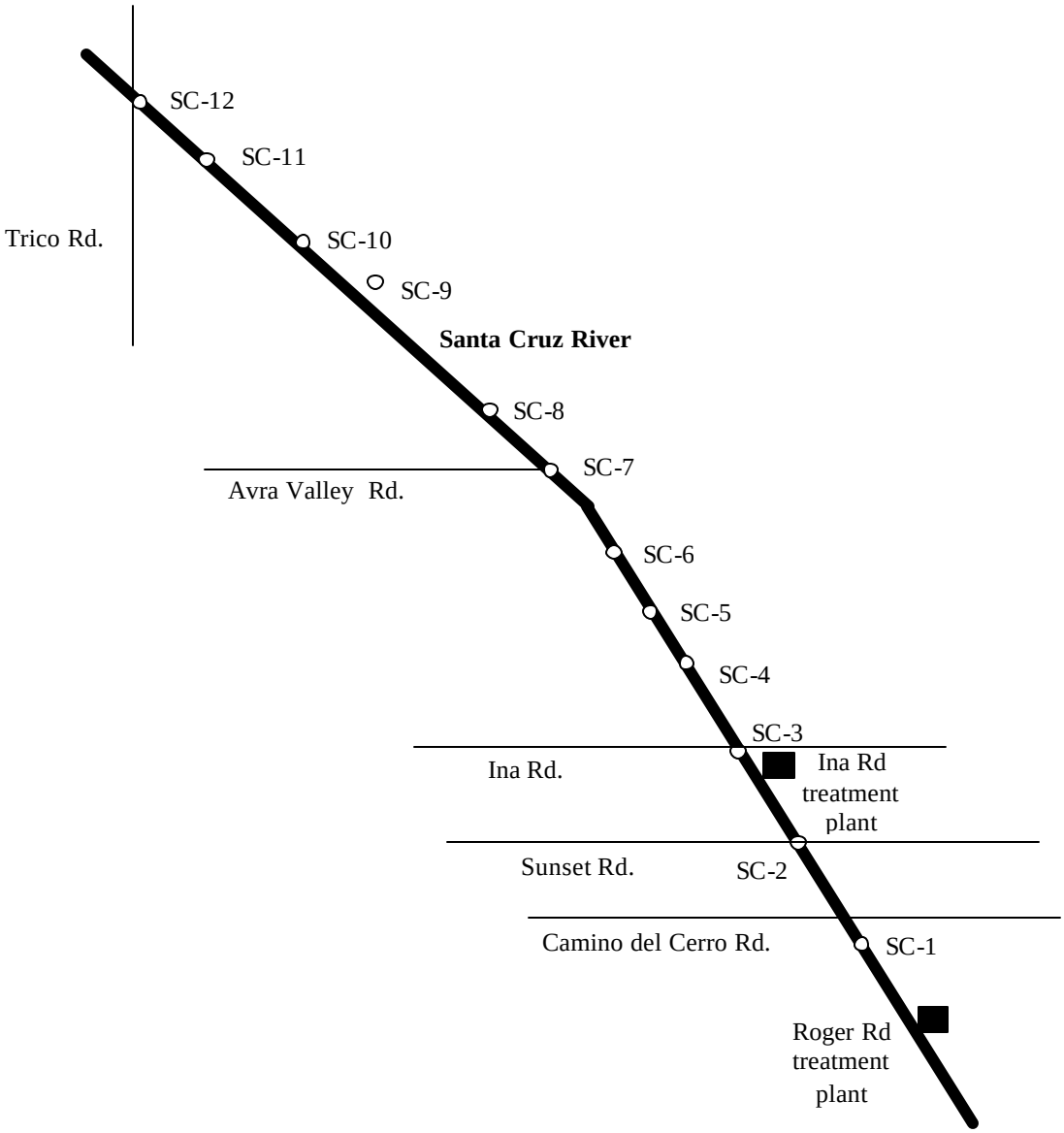

Figure 2. Locations of wells along the Santa Cruz river sampled during this study.

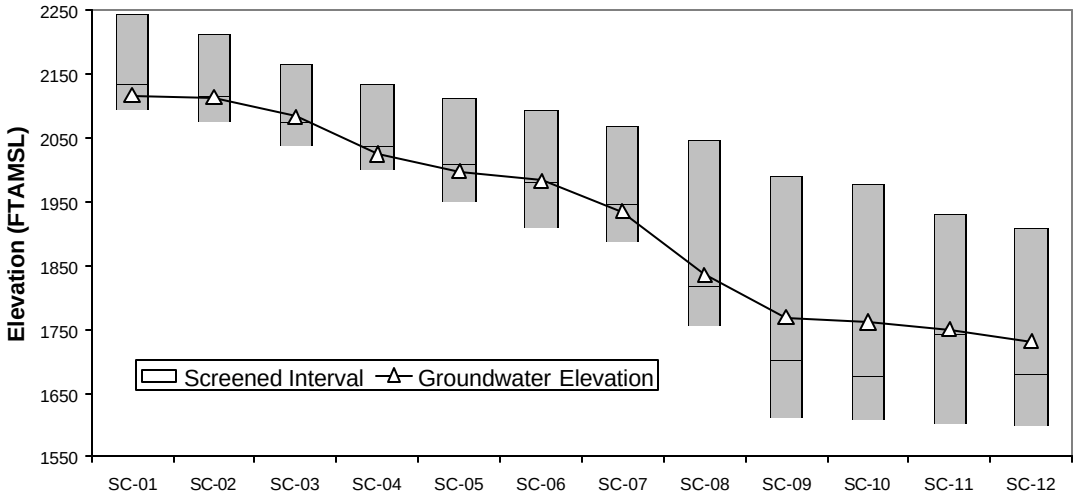

Figure 3. Santa Cruz monitoring well elevations (above mean sea level), screen intervals, and water table elevation. Tops of the lightly shaded portions of each bar represent the local elevation of land surface at the SC-series wells.

Samples for analysis of estrogenic activity were collected in 1-L amber glass bottles that had been acid washed and muffled (550°C). All samples were filtered using 0.45-µm membrane filters (Millipore®) and stored at 4°C pending analysis. Water samples were tested for DOC (dissolved organic carbon), and boron/boron isotopes, as well as for estrogenic activity. Methods for concentrating samples prior to measurement of estrogenic activity are provided in a later section.

Dissolved Organic Carbon. DOC was analyzed using a combustion technique with a TOC-5000 Total Organic Carbon Analyzer. All samples were acidified to pH 2 using 2N HCl2 using 2N HCl and sparged for 4 minutes using ultrapure hydrocarbon-free air, then analyzed 4-6 times to produce a coefficient of variation ≤0.02. Standard solutions (made using potassium hydrogen phthalate) were included in each instrument run, and reported values were derived from a standard curve that was obtained via linear regression analysis. The lower level of detection and practical quantitation limit for DOC measurement with this analyzer were 0.2 and 0.5 mg/L, respectively.

Boron Isotope Ratio. Boron is present as a dissolved constituent in essentially all water sources. Because both the boron concentration and the ratio of stable isotopes $^{11}B/^{10}B$ are variable, waters generally carry a boron signature or "fingerprint." The boron fingerprint in domestic wastewater is frequently very different from that of native ground water (Bassett, 1990; Davidson and Bassett, 1993; Bassett *et al*., 1995). This is particularly useful in groundwater recharge investigations since boron-related measurements (boron concentration and $\delta^{11}B$) can then support estimation of fractional contributions of wastewater and waters from other sources in groundwater samples. Total boron concentration and $^{11}B/^{10}B$ are essentially conserved during wastewater treatment,

infiltration, and subsurface transport and storage of groundwater. In this work, boron isotopes ratio were measured in wastewater effluent and ground waters that were potentially influenced by recharged effluent.

The relative abundances of boron isotopes ^{11}B and ^{10}B are commonly represented by delta B eleven ($\delta^{11}B$) values. The value of $\delta^{11}B$ is calculated using:

$$d\ ^{11}B\ ‰ = \left(\frac{(^{11}B/^{10}B)_{sample} - (^{11}B/^{10}B)_{standard}}{(^{11}B/^{10}B)_{standard}} \right) x\,1000 \qquad (1)$$

where the standard value is based on a specific chemical source, NBS SRM-951. The $^{11}B/^{10}B_{standard} = 4.04362 \pm 0.00137$.

The ratio $^{11}B/^{10}B_{sample}$ was obtained from mass analyses by Thermal Ionization Mass Spectrometry (TIMS), and then compared to a standard ratio corresponding to the National Bureau of Standards Standard Reference Material 951, given above. The value resulting from equation (1) is in units of per mil, ‰.

Based on previous analyses, local wastewater effluent has an average isotopic signature of approximately +2.7‰. Background (ground water) $\delta^{11}B$ values are much higher (+15 to +30‰). The value obtained for each water sample, $d^{11}B_{sample}$, was used to calculate a volume fraction of each end member (source water) by:

$$d^{11}B_{sample} = \frac{\sum_i \left(d^{11}B_i\right)(C_i)(V_i)}{\sum_i (C_i)(V_i)} \qquad (2)$$

where C = total boron concentration
V = endmember volume fractions in the water sample
i = water source (effluent or native ground water)
$d^{11}B_i$ = per mil value of source water i.

The volume fractions of wastewater and groundwater origin in a given sample were determined from equation (2) after measuring $d^{11}B_{sample}$ after assuming the sum of the two volume fractions was equal to one.

Estrogen receptor--b competitor assay. Samples for measurement of estrogenic activity (500-1000 mL) were extracted on C18 disks (Empore, 3M) and eluted using two successive 10-mL washes with reagent-grade methanol. Eluates were combined and evaporated to dryness under nitrogen. Residual organics were resuspended in water containing 0.15 M NaCl to achieve volume concentration factors of 100-200x. Compounds that are efficiently separated from water on C18 disks include natural and synthetic hormones, polychlorinated biphenyls, organochlorine pesticides, phenoxyacid herbicides, phthalates and tirazines—in short, most of the wastewater contaminants that have been implicated in endocrine disrupting activities (manufacturer's data). Previous

work in our laboratory demonstrated that constituents in secondary effluent that are responsible for disruption of ES2/ER-β binding are efficiently removed by passage through the C18 disks (Turney *et al.*, 2002). Process blanks were established using the same concentration/elution procedure.

Analytes for estrogenic activity consisted of unconcentrated well samples or C-18 concentrates in reagent buffer solutions (Pan Vera Corporation). The competitive binding assay is based on displacement of a commercially prepared fluoromone from ER-β. Fluorescence polarization (FP) was used to measure displacement of the fluoromone. Results are frequently reported as the fraction of sample needed to displace 50% of the bound fluoromone from the estrogen receptor (IC_{50}). The competitive-displacement or receptor-binding assay followed procedures established for the Estrogen Receptor-β Competitor Assay developed by the PanVera Company (Bolger *et al.*, 1998). Method detection limits for 17β-estradiol are ~10^{-9} M (Figure 4). The concentration of 17β-estradiol necessary to displace 50% (IC_{50}) of the fluorescent estrogen ligand is about 9 nM. Results were converted to estradiol equivalent (EEQ) concentrations using the results of individual assays (IC_{50} values) and corresponding IC_{50} values derived from positive controls involving 17β-estradiol (Figure 4).

The primary advantages of the competitor assay lie in cost and simplicity. The entire assay can be completed in hours using standard laboratory skills. The procedure detects, but does not differentiate between, agonist (chemicals that stimulate estrogen production) and antagonist (estrogen blocking) compounds. Because the endocrine receptor/fluorochrome complex was directly exposed to the test compounds, without benefit of an intervening cell envelope, test results may not accurately represent the whole-organism response.

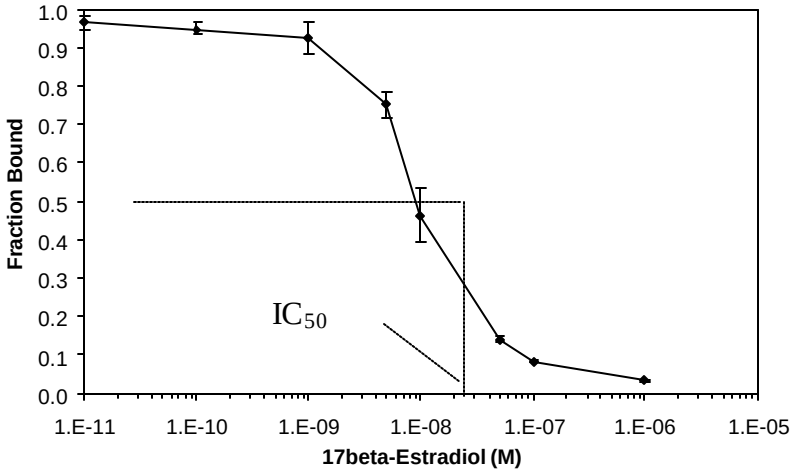

Figure 4. Estrogen receptor-binding assay response for 17β-estradiol. The vertical axis is fraction of the fluorescent ligand bound to the estrogen receptor. Ligand displacement arises from to the presence of competitive (estrogenic) compounds in the sample tested.

C. Principal Findings and Significance

DOC concentrations and estrogenic activities in surface-water samples taken from the Santa Cruz River at positions indicated previously are summarized in Figure 5. It is evident that only modest water quality changes occurred along the 30-mile reach that was sampled. Boron isotope ratios indicated that waters of the Santa Cruz were entirely of wastewater origin (data not shown) over the length monitored. Estrogen activity measurements were typical of wastewater effluents from the Roger Road and Ina Road Wastewater Treatment Plants, as indicated by previous work (results not shown). Furthermore, estrogenic activity showed little evidence of attenuation with distance traversed or time of travel in the river. Bulk organic levels were similarly conserved. Dry weather flow in the Santa Cruz River below the Roger Road and Ina Road outfalls is typically shallow, from just inches to several feet in depth. Because dry weather travel times along the reach sampled are on the order of hours to days, it is concluded that sunlight, including its ultraviolet component, has little effect on estrogenic compounds in conventionally treated secondary effluents. Similarly, biodegradation does not seem to have a dramatic effect on estrogenic activity or bulk organic composition over periods of days in surface waters. Since the samples were taken in June 2001, during which the average daily temperature was 85.6° F (NOAA), it is unlikely that biochemical activity in the river has a greater effect during another time of year.

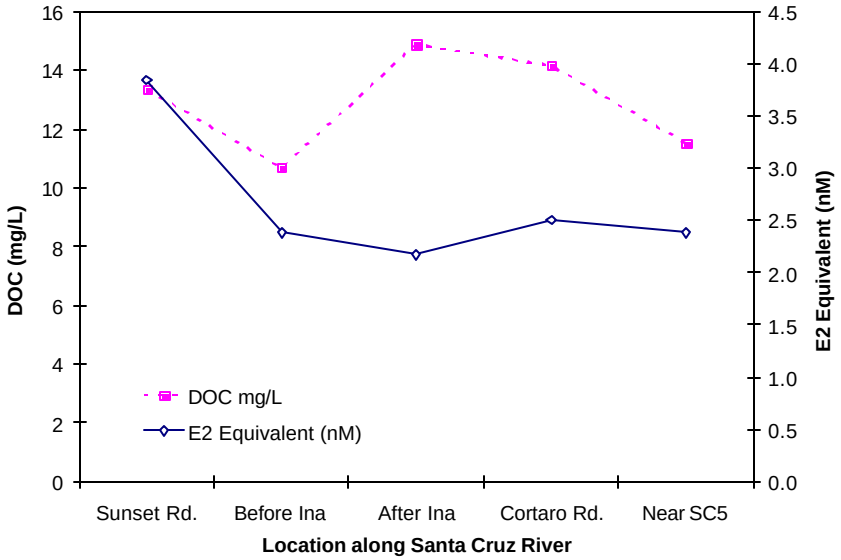

Figure 5. DOC concentrations and estrogenic activities in surface-water samples taken from the Santa Cruz River.

Water quality data for the Santa Cruz series wells (SC-01 through SC-12) show considerable spatial variation (Figure 6). At least three of the shallow (relative to the groundwater table) wells were dominated by water of wastewater origin—SC-01, SC-03 and SC-06. Somewhat unexpectedly, waters from the intervening wells SC-02, SC-04 and SC-05 indicated that the volume fraction of wastewater origin was no greater than

about 0.30. Overall, results suggest that waters in as least some of the Santa Cruz series wells consist of a mixture of effluent and native ground water. The sizable contribution of native ground water may originate from water use/disposal activities at the surface, such as gravel washing operations. Alternatively, waters from wells with only modest effluent components may simply be diluted with native ground water at points of particularly high permeability and subsurface flow.

The tri-linear diagram (Figure 6) shows two very distinct water types and one intermediate. The first main grouping includes SC-01 and SC-02, which are similar to SC-03 through SC-07. Monitor well data for SC-08 appears to be an intermediate between the two groupings and SC-09 through SC-12 make up the second grouping. SC-02 lies directly over SC-01 so it is not shown for clarity. This is also true for SC-04 through SC-07, which plot over SC-3, and SC-10 through SC-12 which plot over SC-09. The Rillito Narrows (near Avra Valley Road and well SC-07) separates the Upper Santa Cruz sub-basin from the Avra Valley sub-basin. Monitoring wells SC-9 through SC-12 lie in the Avra Valley sub-basin. There is a large increase in depth to groundwater north of the narrows (Figure 3).

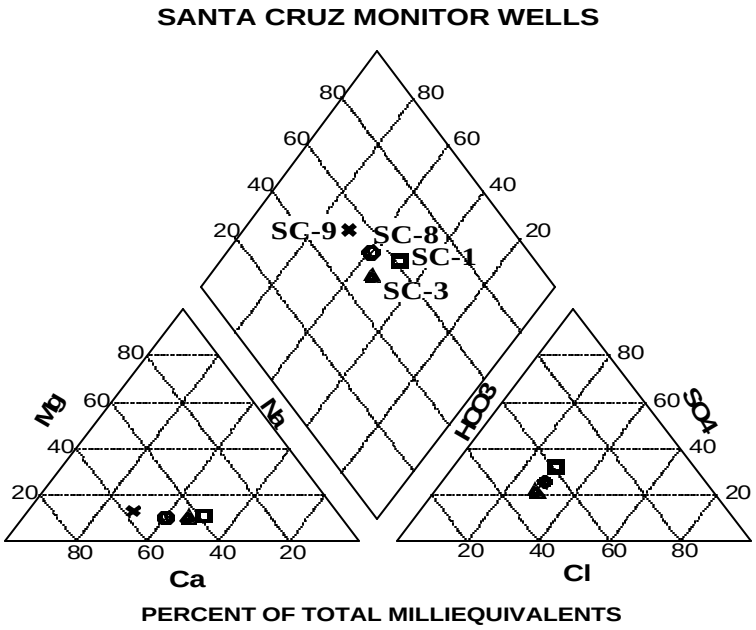

Figure 6. Tri-linear diagram for the Santa Cruz series wells (SC-01 through SC-12).

None of the wells downstream (north) of SC-07 was much affected by infiltrating wastewater effluent, suggesting that local infiltration or groundwater flow patterns in that region transport effluent away from those sampling points.

Estrogenic activities (in units of equivalent E2 concentrations) were highly correlated (r^2 = 0.92) to fractional wastewater content in well samples (Figure 7). That is, wells that

contained the highest volume contributions of wastewater generally showed the highest estrogenic activity. Wells further north (downstream) than SC-07 contained waters at or near our detection limit for estrogenic activity. Those that were dominated by effluent produced E2 equivalent concentrations that were comparable to those in Santa Cruz surface waters. Results suggest that little attenuation of compounds contributing to measured estrogenic activity occurred during infiltration from the surface to well withdrawal points and/or subsequent storage as ground water. Well SC-01 offers the lone contradictory data point. Boron isotopes indicate that this water is entirely of wastewater origin. Nevertheless, estrogenic activity and DOC concentration (see below) were low. The anomalous result cannot be rationalized without additional monitoring work.

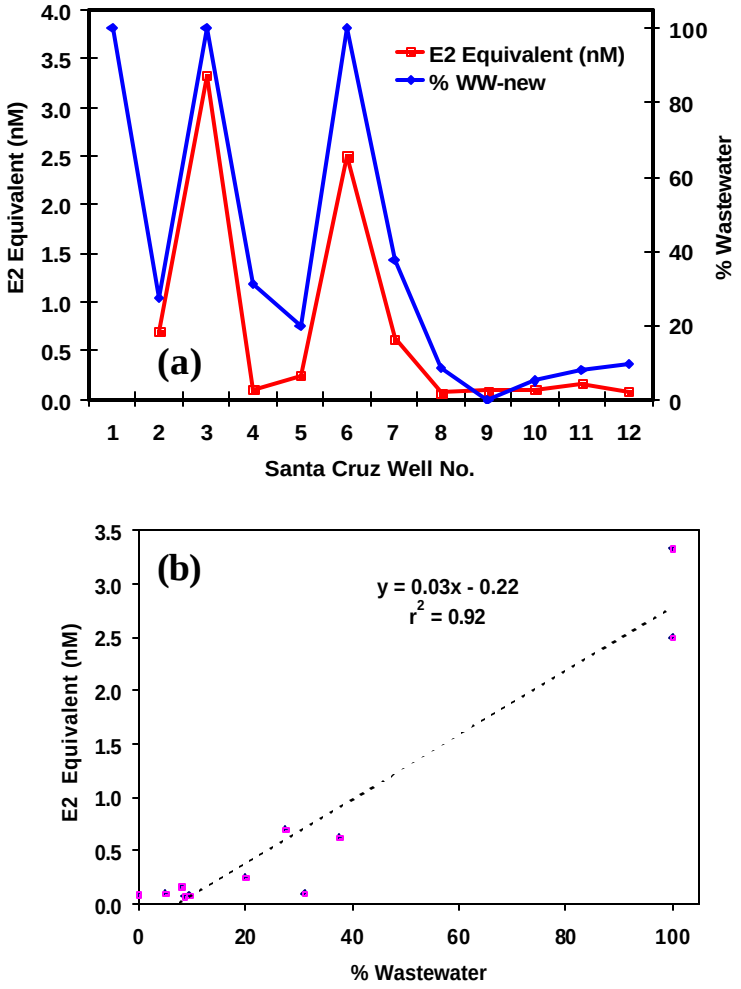

Figure 7. Estrogenic activity (in units of E2 equivalent concentration) and fractional wastewater content in SC well samples (a) and regression analysis illustrating the dependence of estrogenic activity on volume wastewater contribution in the samples (b).

DOC concentrations were also strongly related to boron isotope data ($P < 0.0003$) (Figure 8). DOC concentrations in the Santa Cruz well series never exceeded 2.8 mg/L and were significantly above 2.0 mg/L in just one sample. This is in contrast to DOC values in plant effluent (typically 12-15 mg/L) and values in Santa Cruz River water (10-14 mg/L)

during the study. From DOC values in SC-01, SC-03 and SC-06, it is evident that bulk organic residuals in Roger Road and Ina Road Wastewater Treatment Plant effluents are further attenuated during infiltration and storage as ground water. Compounds that contribute to the measured estrogenic activity are not similarly affected by infiltration and storage.

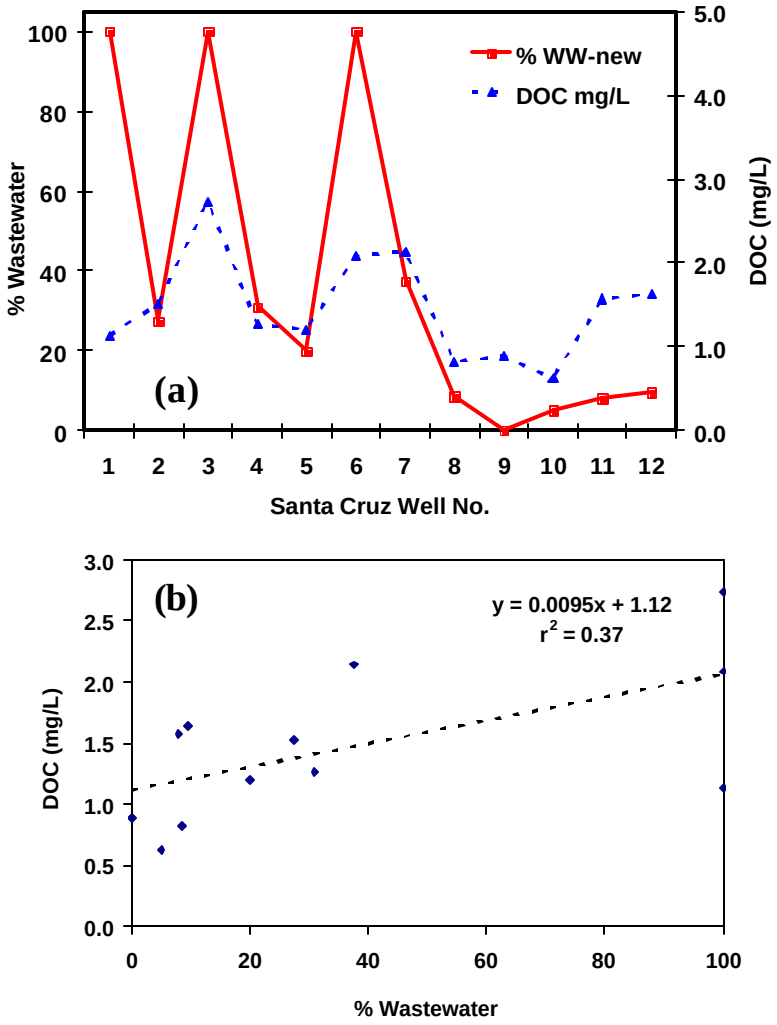

Figure 8. DOC concentration and boron isotope data for SC well samples (a) and regression analysis showing the dependence of DOC concentration on fractional (volume) contribution to the sample (b).

In contrast to these results, previous work (Turney *et al*., 2002) showed that both DOC concentration and estrogenic activity are efficiently removed during the managed infiltration of wastewater effluent for ground water replenishment. In that study, DOC was typically reduced by >90 percent and estrogenic activity (competitive displacement assay, as here) was lowered by a factor of about 20 during managed infiltration through about 100 feet of unconsolidated sediment. Infiltration basins were operated by alternating wet and dry periods that were several days in length. Such basins typically develop a biochemically active surface layer (schmutzdecke) that is disproportionately

important to organic attenuation processes. Among the potentially important differences between managed and incidental (in-stream) recharge is the continuous maintenance of a schmutzdecke in zones where most of the water infiltrates.

Conclusions and Recommendations

Results of the study support the following conclusions and recommendations for follow-on work.

i. When surface water samples were taken from the Santa Cruz River, the entire reach sampled was dominated by reclaimed water. There were no other major water contributors to the river over the 30-mile region sampled.

ii. There was little or no evidence of attenuation of estrogenic activity with distance traveled among surface water samples. Since the river is fairly shallow at most points and times of travel in the reach sampled are on the order of hours to days, it is likely that solar irradiation plays a modest or inconsequential role in transforming compounds that are responsible for residual estrogenic activity in reclaimed water.

iii. At several points along the river, shallow ground waters are strongly affected by reclaimed water. The bulk of water samples derived from three wells (SC-01, SC-03, and SC-06) were of wastewater origin, as indicated by measurements of boron isotope ratios. Much lower volume contributions of reclaimed water at intervening wells SC-02, SC-04, and SC-05 indicate that mixing processes arising from water use patterns at land surface (e.g. gravel washing using well water) or subsurface dilution with native ground water also contributes to groundwater quality characteristics at these locations.

iv. There is a strong relationship between the fractional (volume) contribution of reclaimed water and estrogenic activity in the SC well series. There is a weaker, yet significant relationship between DOC concentration and estrogenic activity in the same set of samples. Results suggest that water quality in shallow wells at some locations along the Santa Cruz River may be affected by the infiltration of reclaimed water from the Roger Road and Ina Road Wastewater Treatment Plants.

v. In the Santa Cruz wells with large volume contributions of reclaimed water, estrogenic activity and DOC concentrations were high relative to values in wells dominated by reclaimed water at the Sweetwater Recharge Facilities. These findings suggest that managed infiltration of wastewater effluent can produce water quality benefits that are not realized, at least not immediately, during the unmanaged, incidental recharge of reclaimed water in a river bed.

vi. Although study results present a fairly consistent picture, data from SC-01 are anomalous in that boron isotopes indicate that the water is dominated by effluent, but measures of both DOC and estrogenic activity are in the low range of values encountered in the study. Some additional thought and/or monitoring is necessary to clear up this apparent inconsistency.

vii. The competitive-displacement assay for estrogenic activity should be backed up by a more physiologically interpretable assay in subsequent work—at least until

reliable correlations among the results of different assays for complex water samples are available.

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